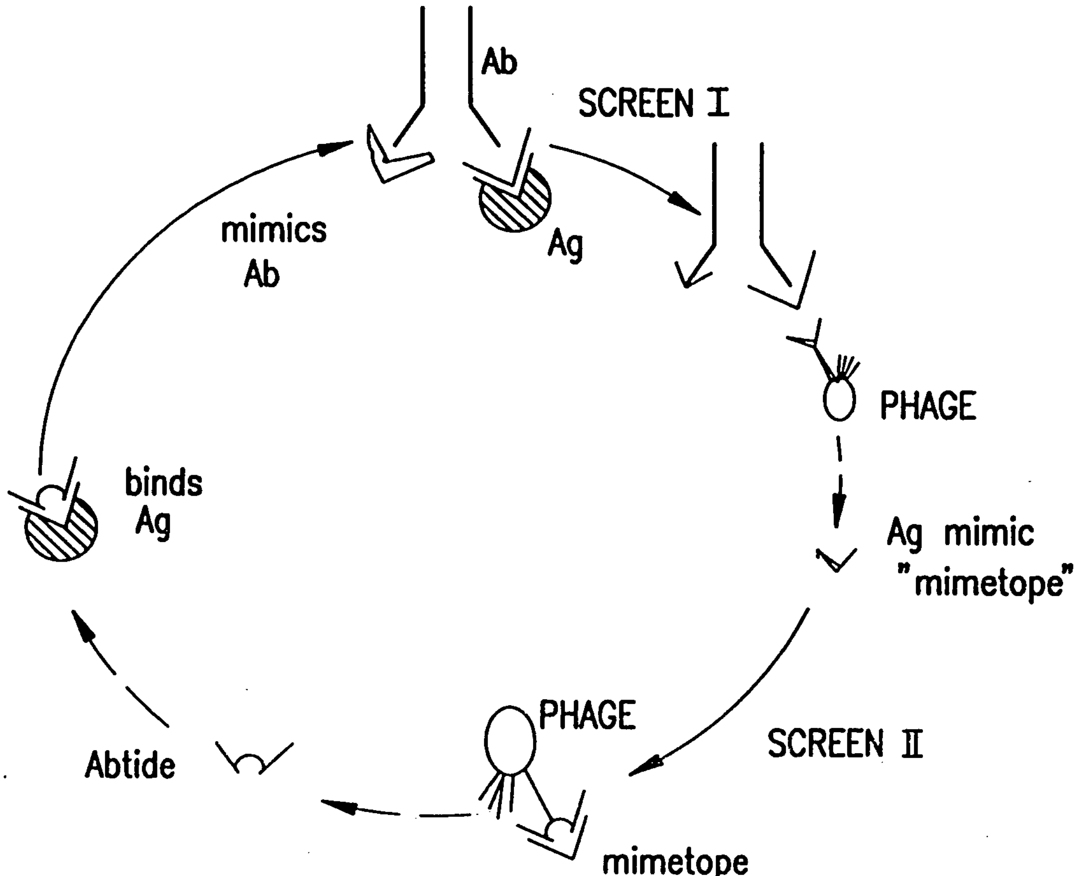

FIG.1

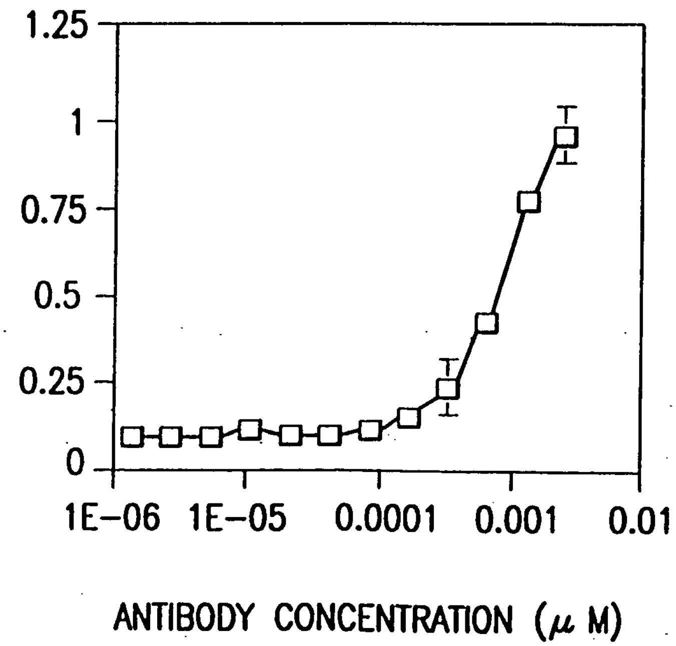

FIG.2

CLONE 14

GIINANDPLPFWFMS--PYTPGPAPIDINASRALVS-NESG

WQGTHFPYT — CDR3L — (5/9 = 55.5 %)

LVSKNDSG — CDR2L — (7/8 = 87.5 %)

CLONE 17

DL-SRNLDFGRFLLYNA--YVPGFTPTFISLTAEHLSSPKG

LVSKN-DSG — CDR2L — (6/8 = 75 %)

WQGTHF-P-YT — CDR3L — (6/9 = 66.6 %)

CLONE 15

CGRAYCL-SGNYNIFGALFPGVS--TPYADVGHDDAQSWRR

LVSKN-DS-G — CDR2L — (4/8 = 50 %)

WQG-THFPYT — CDR3L — (6/9 = 66.6 %)

CLONE 13

RCSPIW-GIS-YPFGLLSSNPGVCHSSDAET-NIRNDILTT

WQG-THFPYT — CDR3L — (6/9 = 66.6 %)

GSDN-K-SVL — CDR2L(REV) — (4/8 = 50 %)

CLONE 16

GHSNYCFVSTLGMPIVGFP-SINARGLIHYGGSDPR--LAA

WQGTHFPYT — CDR3L — (3/9 = 33.3 %)

GSDNKSVL — CDR2L(REV) — (5/8 = 62.5 %)

FIG.3

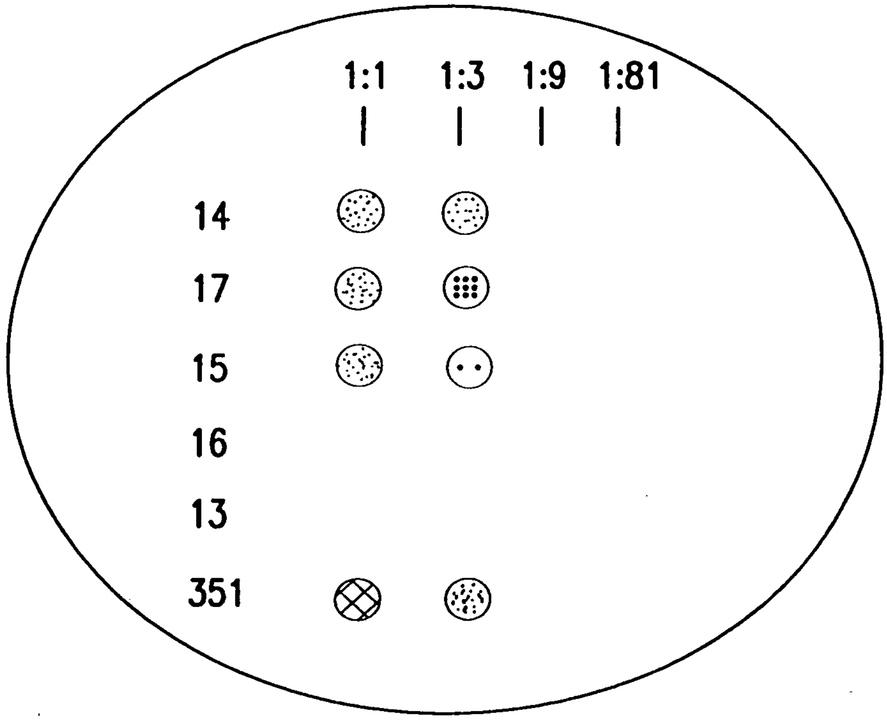

FIG.4

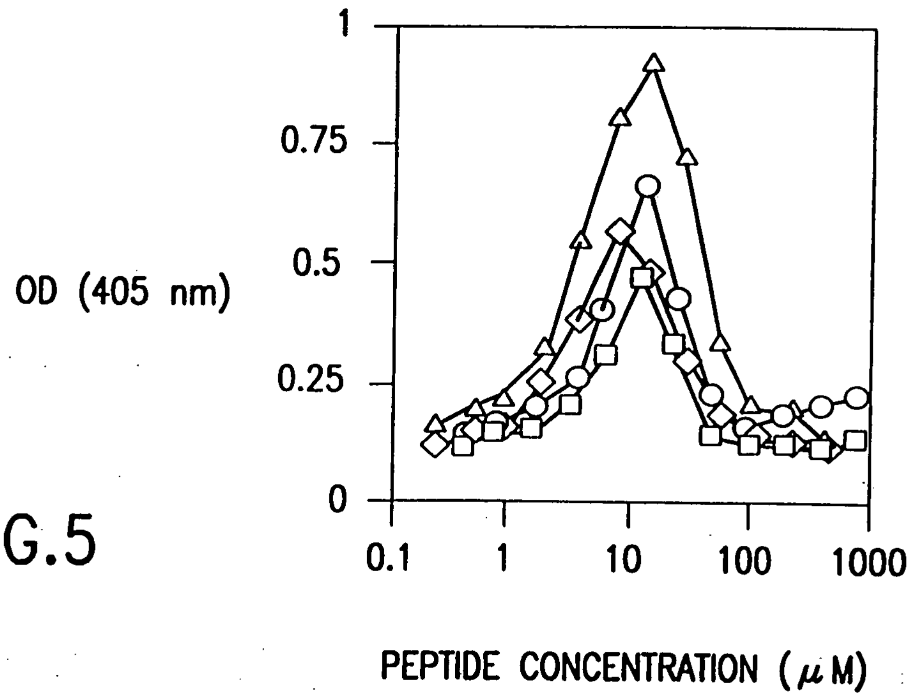

FIG.5

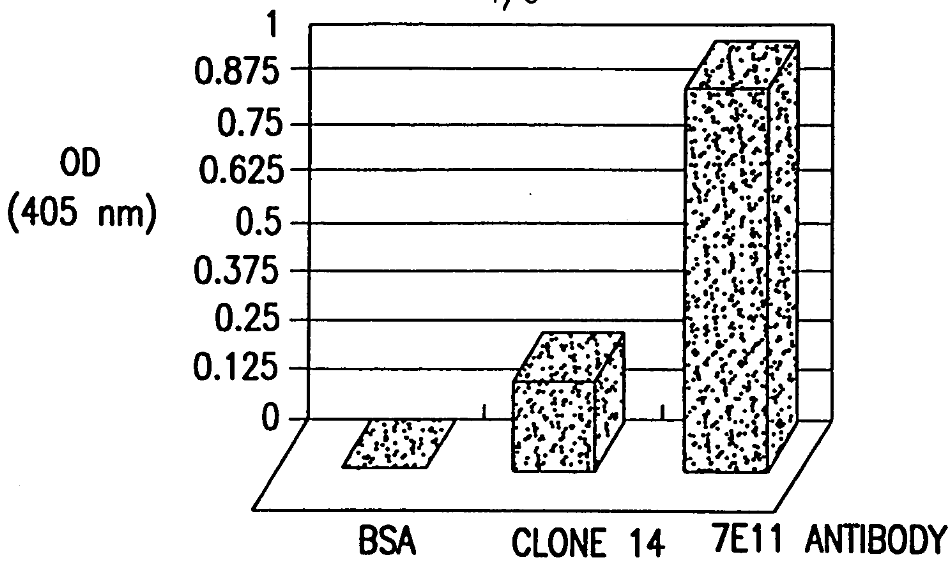

FIG.6

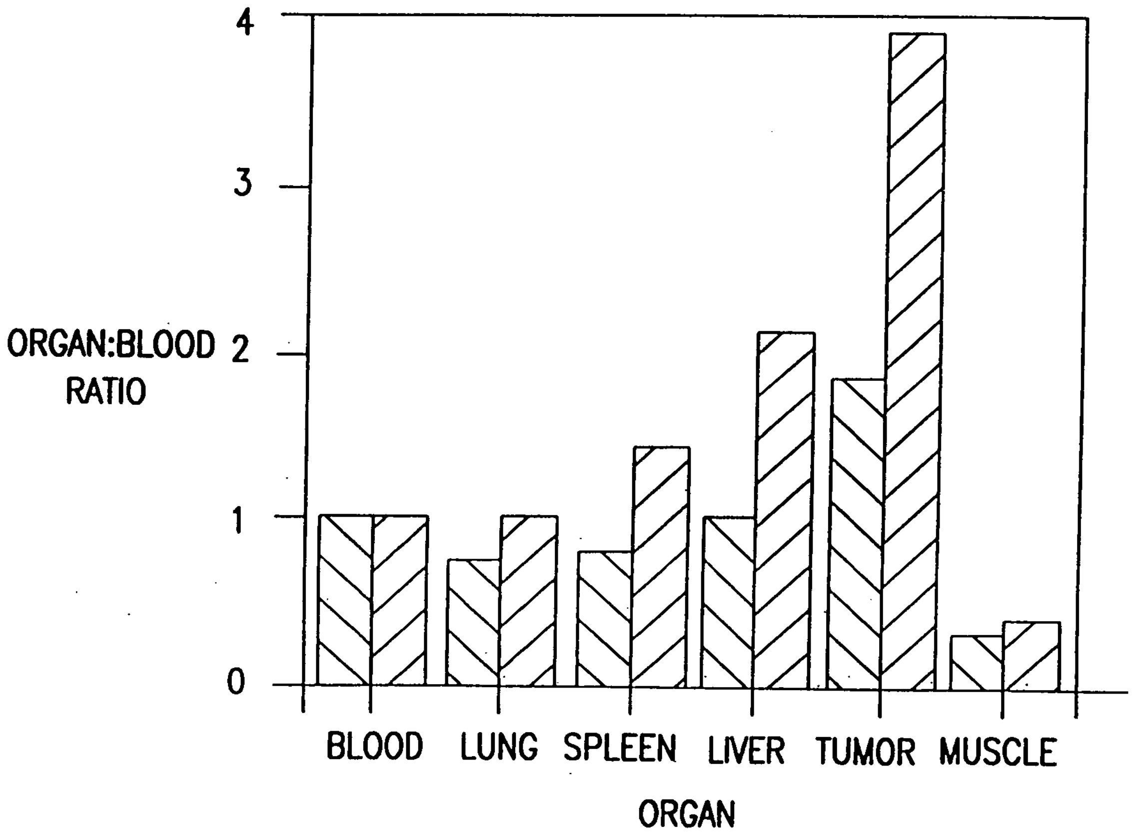

FIG.7

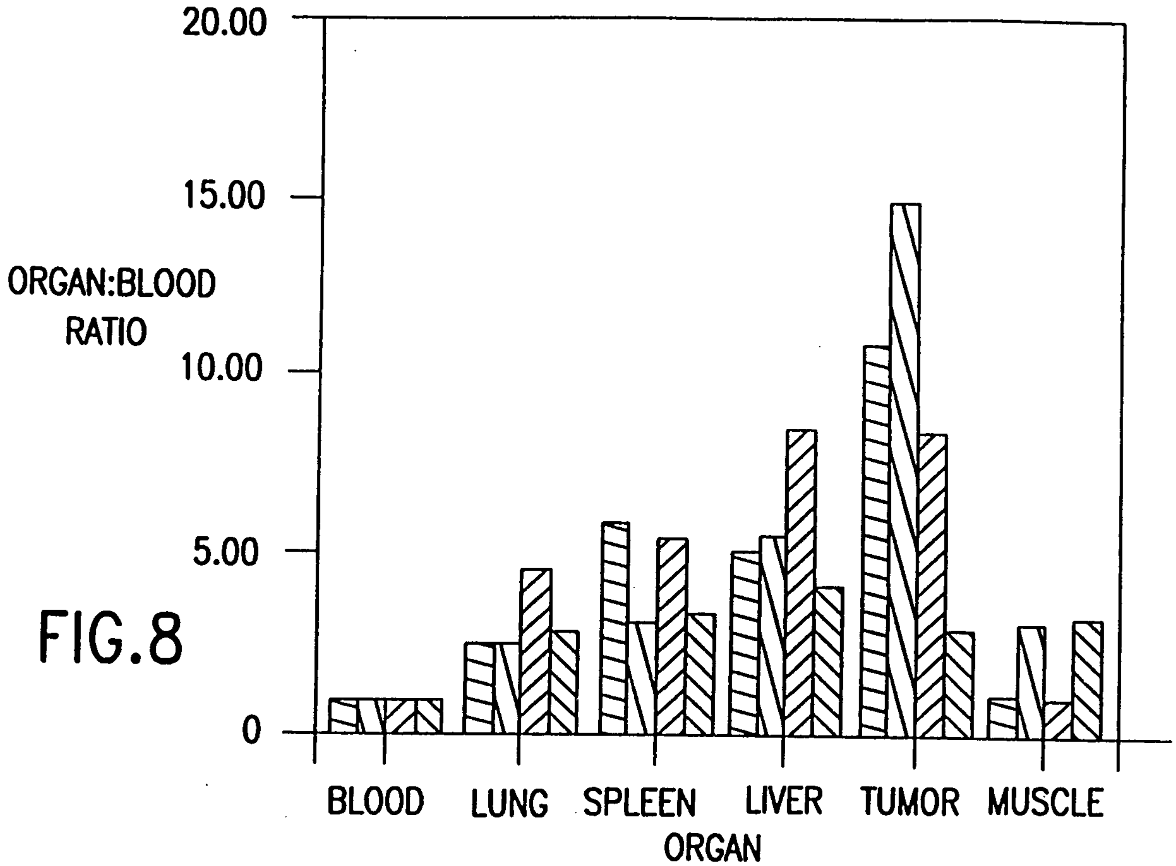

FIG.8

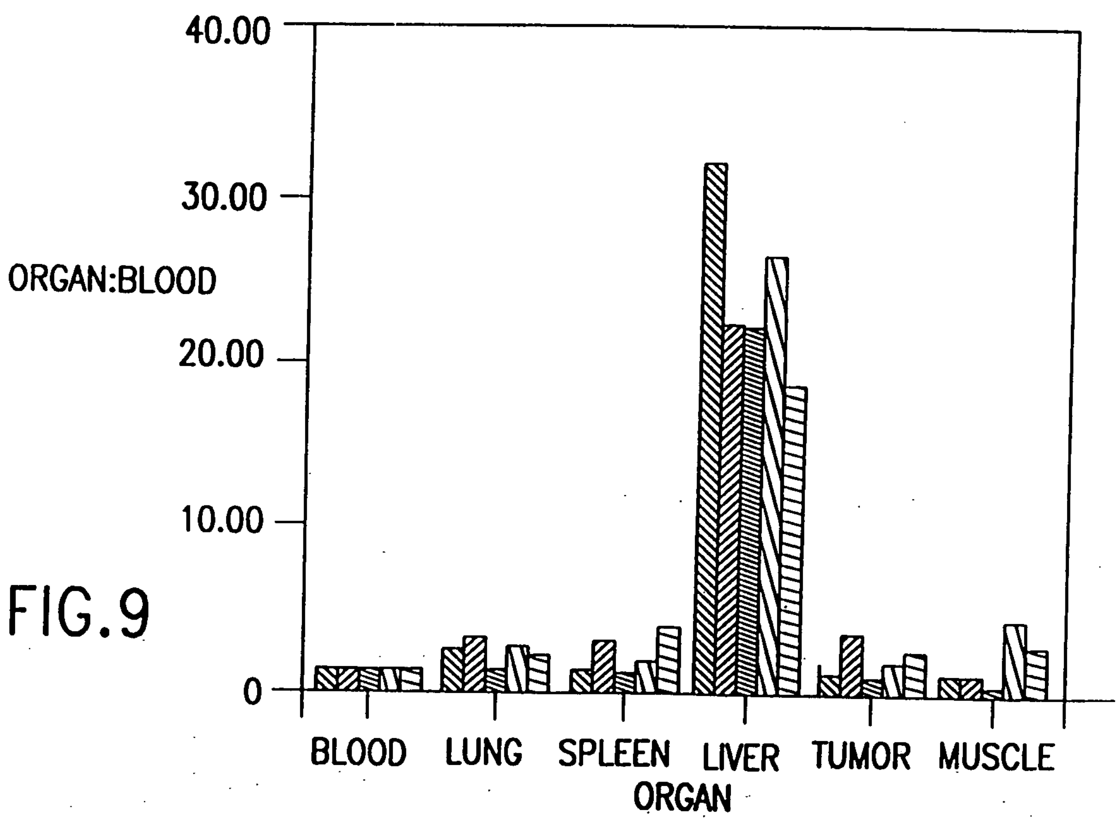

FIG.9

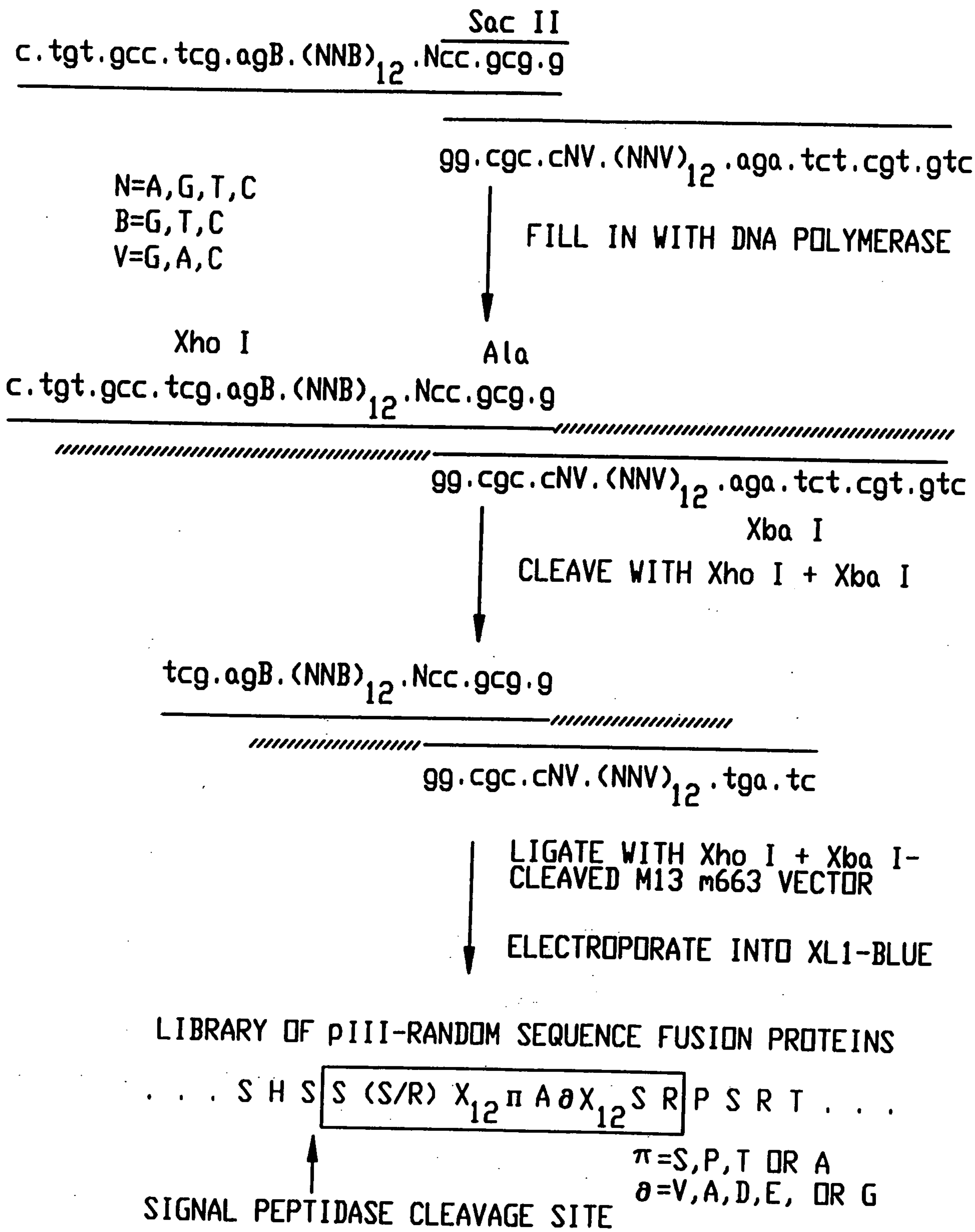
Sac II
c.tgt.gcc.tcg.agB.(NNB)12.Ncc.gcg.g
gg.cgc.cNV.(NNV)12.aga.tct.cgt.gtc
N=A,G,T,C
B=G,T,C
V=G,A,C
FILL IN WITH DNA POLYMERASE
Xho I
Ala
c.tgt.gcc.tcg.agB.(NNB)12.Ncc.gcg.g
gg.cgc.cNV.(NNV)12.aga.tct.cgt.gtc
Xba I
CLEAVE WITH Xho I + Xba I
tcg.agB.(NNB)12.Ncc.gcg.g
gg.cgc.cNV.(NNV)12.tga.tc
LIGATE WITH Xho I + Xba I-
CLEAVED M13 m663 VECTOR
ELECTROPORATE INTO XL1-BLUE
LIBRARY OF pIII-RANDOM SEQUENCE FUSION PROTEINS
. . . . S H S S (S/R) X12 π A ∂ X12 S R P S R T . . .
π=S,P,T OR A
∂=V,A,D,E, OR G
SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.10

G TGT GTC TCG AGN $(NNB)_{20}$ NAC GCC AN

NTG CGG TNV $(NNV)_{15}$ AGA TCT GTG TTG

N=A,C,G,T
B=C,G,T
V=A,C,G

↓ FILL IN WITH SEQUENASE

Xho I

G TGT GTC TCG AGN $(NNB)_{20}$ NAC GCC AN

NTG CGG TNV $(NNV)_{15}$ AGA TCT GTG TTG
Xba I

↓ RESTRICT WITH Xho I AND Xba I

TCG AGN $(NNB)_{20}$ NAC GCC AN

NTG CGG TNV $(NNV)_{15}$ AGA TC

↓ LIGATE WITH Xho I + Xba I-CLEAVED M13mp18Xa

↓ ELECTROTRANSFORM E. coli JS5

D38 GENETIC DIVERSITY LIBRARY DISPLAYED AS RANDOM N-TERMINAL pIII FUSIONS

. . H S S (S/R) X_{20} (Y/H/N/D) A (I/M/T/N/K/S/R) X_{15} S R

↑ SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.11

G TGT GTC TCG AGN $(NNB)_{20}$GGT TGT GGT

CCA ACA CCA $(NNV)_{20}$AGA TCT GTG TTG

N=A,C,G,T
B=C,G,T
V=A,C,G

↓ FILL IN WITH SEQUENASE

Xho I

G TGT GTC TCG AGN $(NNB)_{20}$GGT TGT GGT

CCA ACA CCA $(NNV)_{20}$AGA TCT GTG TTG

Xba I

↓ RESTRICT WITH Xho I AND Xba I

TCG AGN $(NNB)_{20}$ GGT TGT GGT

CCA ACA CCA $(NNV)_{20}$AGA TC

↓ LIGATE WITH Xho I + Xba I-CLEAVED M13mp18Xa

ELECTROTRANSFORM *E. coli* JS5

DC43 GENETIC DIVERSITY LIBRARY DISPLAYED AS RANDOM N-TERMINAL pIII FUSIONS

. . H S S (S/R) X_{20} G C G X_{20} S R

↑ SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.12

G A P V W R G N P R W R G P G G F K W P G C G N G P M C N T F T P A R G G S R N N G P

5' ggs-gcs-ccs-gts-tgs-ags-ggs-aas-ccs-cgs-tgs-ags-ggs-ccs-ggs-ggs-tts-aas-tgs-ccs-GGC-TGC-GGG 3'

MP-1 oligo 1

3' CCG-ACG-CCC-tts-ccs-ggs-tas-acs-tis-tgs-aas-tgs-ggs-cgs-gcs-ccs-ccs-ags-gcs-tts-tts-ccs-ggs 5'

MP-1 oligo 2

FIG.13